Applied and Environmental Microbiology, Oct. 2010, p. 6829–6836 Vol. 76, No. 20
0099-2240/10/$12.00 doi:10.1128/AEM.01630-10

Coexistence of Pathogens in Host-Seeking and Feeding Ticks within a Single Natural Habitat in Central Germany▽†

Jan Franke,[1]* Julia Fritzsch,[2] Herbert Tomaso,[3] Eberhard Straube,[2] Wolfram Dorn,[1] and Anke Hildebrandt[2]

Institute of Nutrition, Department of Food and Environmental Hygiene, Friedrich Schiller University, Jena, Germany[1]; Medical University Laboratories, Institute of Medical Microbiology, Friedrich Schiller University, Jena, Germany[2]; and Federal Research Institute for Animal Health, National Reference Center for Tularemia, Jena, Germany[3]

Received 8 July 2010/Accepted 13 August 2010

The importance of established and emerging tick-borne pathogens in Central and Northern Europe is steadily increasing. In 2007, we collected *Ixodes ricinus* ticks feeding on birds ($n = 211$) and rodents ($n = 273$), as well as host-seeking stages ($n = 196$), in a habitat in central Germany. In order to find out more about their natural transmission cycles, the ticks were tested for the presence of Lyme disease borreliae, *Anaplasma phagocytophilum*, spotted fever group (SFG) rickettsiae, *Francisella tularensis*, and babesiae. Altogether, 20.1% of the 680 ticks examined carried at least one pathogen. Bird-feeding ticks were more frequently infected with *Borrelia* spp. (15.2%) and *A. phagocytophilum* (3.2%) than rodent-feeding ticks (2.6%; 1.1%) or questing ticks (5.1%; 0%). *Babesia* spp. showed higher prevalence rates in ticks parasitizing birds (13.2%) and host-seeking ticks (10.7%), whereas ticks from small mammals were less frequently infected (6.6%). SFG rickettsiae and *F. tularensis* were also found in ticks collected off birds (2.1%; 1.2%), rodents (1.8%; 1.5%), and vegetation (4.1%; 1.6%). Various combinations of coinfections occurred in 10.9% of all positive ticks, indicating interaction of transmission cycles. Our results suggest that birds not only are important reservoirs for several pathogens but also act as vehicles for infected ticks and might therefore play a key role in the dispersal of tick-borne diseases.

Lyme borreliosis is the most frequent arthropod-borne disease in the northern hemisphere (6), but other pathogens, such as intracellular bacteria of the order *Rickettsiales* (*Anaplasma phagocytophilum*, spotted fever group [SFG] rickettsiae), *Francisella tularensis*, and intraerythrocytic parasites of the genus *Babesia*, have gained more and more importance as tick-borne agents in Europe (52). The castor bean tick (*Ixodes ricinus*) has a three-host life cycle, which means that it ingests a blood meal in each life stage before it molts. When transovarial transmission of a pathogen is absent or very rare, as is the case with *Borrelia* spp. (63), *A. phagocytophilum* (10), and *Babesia microti* (15), detection of these agents in feeding larvae is an indication of pathogen transmission from an infected reservoir host to the tick. With the exception of SFG rickettsiae, which use ticks as the vector and reservoir, established and emerging pathogens are maintained by vertebrate reservoirs during their life cycles. Although methods for detection and characterization are constantly improving, the ecology of tick-borne pathogens, particularly their reservoir host specificity, is still not understood in detail.

The agents of Lyme disease form a very heterogeneous complex, which can be subdivided into several clusters by phylogenetic analysis of genes (e.g., *ospA*) or noncoding regions. Several bird, rodent, and reptile species act as reservoirs for these spirochetes (32).

Anaplasma phagocytophilum is the causative agent of human granulocytic anaplasmosis (HGA), an influenza-like illness of humans and domestic animals which is widespread in Europe. Sheep, deer, and rodents have been discussed as reservoir hosts for HGA agents (28, 31). Birds might be of importance in the dispersal of *Anaplasma*-infected ticks over long distances (18).

At least half of the about 30 SFG rickettsiae distributed worldwide that have been described so far are known to be pathogenic for humans. Because efficient transovarial transmission of SFG rickettsiae from female ticks to larvae has been described for several species, e.g., *Rickettsia parkeri*, *R. slovaca*, and *R. helvetica*, the tick vector can also be regarded as a reservoir host (38). Some small mammals, like meadow voles and chipmunks, develop a strong rickettsemia which might allow transmission to parasitizing ticks (38). Although a vehicle function of birds is hypothesized (13), further investigations are needed to ascertain their possible role as reservoir hosts for SFG rickettsiae.

Tularemia is a zoonotic disease caused by *F. tularensis*. In Germany, only the subspecies *F. tularensis* subsp. *holarctica* is prevalent, primarily in wild mammals (lagomorphs and rodents), but humans can become infected through the bite of hematophagous arthropods, by direct contact with infected animals (mostly hares), by ingestion of contaminated food or water, or by inhalation of infected aerosols (57). Potential vectors include ticks, mosquitos, and deer flies (40). However, in Germany, only ticks seem to play a relevant role. Movements of birds that excrete the bacteria with their feces might explain the transfer to islands and over long distances (35).

Besides bacterial and viral agents, pathogenic parasites are also transmitted by *I. ricinus*. Protozoa of the genus *Babesia*

* Corresponding author. Mailing address: Department of Food and Environmental Hygiene, Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Str. 29, 07743 Jena, Germany. Phone: 49-3641-949694. Fax: 49-3641-949602. E-mail: ja.frae@uni-jena.de.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 20 August 2010.

invade erythrocytes and cause an often life-threatening malaria-like disease in humans and animals. Rodents are frequently infected with *Ba. microti*, but there is still no final evidence for a reservoir role of small mammals for pathogenic *Babesia* species. The main reservoirs of *Babesia divergens* are cattle and deer (15). Recently, we discovered that bird-feeding subadult ticks are frequently infected with *Ba. divergens*- and *Ba. microti*-like species, indicating an important role of migratory passerines as reservoirs and in the dispersal of *Babesia* spp. (18).

The aim of the present study was to gain information about the cocirculation of five tick-borne pathogens in a single natural focus, especially regarding their preferred reservoir hosts and vehicles.

MATERIALS AND METHODS

Trapping. From May to October 2007, feeding and questing ticks were collected in two investigation areas in Reifenstein (Thuringia), a village in central Germany (51°20′54.65″N, 10°21′38.74″E). Both areas are located next to each other and are separated only by a country road. The main area is about 15 ha in size and consists of a beech grove, a residual alluvial forest with a marsh area, and agricultural crop land containing two sedimentation ponds with reeds. The second area is about 8 ha in size and contains a small pond with reeds and sedges, a well-developed field layer, an understory, and deciduous trees.

Birds were captured with nets by a professional ornithologist during regular ringing work. Small mammals were captured with daily-controlled live traps (Grahnab, Gnosjö, Sweden). Birds and rodents were released after removal of semiengorged to almost fully engorged ticks. Data for hosts that were not infested with engorged ticks were not included in the present study. Additionally, questing ticks were captured by flagging. All ticks were transported on ice to the laboratory and stored at −80°C until analysis.

Sample preparation and screening. DNA extraction was performed with a GenElute bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO). All samples were screened for microbial DNA by PCR, using the following pathogen-specific targets: *Borrelia ospA* (29), *A. phagocytophilum* 16S rRNA gene (21), *Rickettsia gltA* (36), *F. tularensis Tul4* and *ISFtu2* (59), and *Babesia* 18S rRNA gene (17). For *Borrelia*-specific PCR, HotMaster *Taq* (VWR International GmbH, Darmstadt, Germany) was used. Detection of *A. phagocytophilum*, rickettsiae, and babesiae was performed with TaKaRa EX *Taq* (Takara Bio, Ōtsu, Japan). *Francisella tularensis* was detected via TaqMan real-time PCR, as described previously (59). Reactions were performed under a laminar-flow cabinet with sterile techniques. Due to the limited volumes of extracted DNA, the number of samples analyzed varied between the different pathogens.

Validation and species identification. To identify *Borrelia* genospecies, a restriction fragment length polymorphism (RFLP) method, using FastDigest endonucleases (Fermentas, St. Leon-Rot, Germany), has been applied, as described previously (29). For sequencing, gene fragments were extracted from agarose gels using an agarose gel extraction kit (Jena Bioscience, Jena, Germany) and analyzed following a standard protocol (DYEnamic ET Terminator cycle sequencing kit; GE Healthcare, Chalfont St. Giles, United Kingdom). The sequences were compared with those of reference strains from the NCBI database (http://blast.ncbi.nlm.nih.gov/; see Table S1 in the supplemental material), using ClustalX software for multiple-sequence alignments and bootstrap iteration (58). Not all *Rickettsia*-positive samples were identified to the species level. The main objective of sequencing was the verification of weak *Rickettsia*-positive amplicons. Because *Ba. divergens* and *Ba. microti* are actually complexes of several closely related species, sequence analysis was applied only to discriminate between *Ba. divergens*- and *Ba. microti*-like species. Statistical analysis was performed with Fisher's exact test and Student's *t* test, and a *P* value of ≤0.05 was considered statistically significant.

RESULTS

Host species and tick infestation. A total of 680 ticks (326 larvae, 318 nymphs, 36 adults) have been collected off vertebrate hosts and vegetation (Tables 1 to 3). All birds included in the present study are ground-foraging or ground-dwelling spe-

TABLE 1. Infection rates and species classification of tick-borne pathogens in bird-feeding ticks collected in Reifenstein (central Germany) from May to October 2007

Source	*Borrelia* spp.[a]												*Rickettsia* spp.			*Babesia* spp.				
	Prevalence (% [no. positive/no. tested])	No. of infected larvae/nymphs[c]											Prevalence (% [no. positive/no. tested])	No. of infected larvae/nymphs[c]		Prevalence (% [no. positive/no. tested])	No. of infected larvae/nymphs[c]			Prevalence of *F. tularensis* (% [no. positive/no. tested])[b]
		Bo. burgdorferi	*Bo. afzelii*	*Bo. bavariensis*	g3	g5	g6	g8	vI	vII	NI[d]	*A. phagocytophilum* (% [no. positive/no. tested])[b]		*R. monacensis*	*R. helvetica*		*Ba. microti*	*Ba. divergens*	NI	
Feeding ticks	15.2 (32/211)											3.2 (6/189)	2.1 (4/189)			13.2 (25/189)				1.2 (2/180)
Larvae	20.6 (14/68)											3.2 (2/63)	(0/63)			19.0 (12/63)				(0/61)
Nymphs	12.6 (18/143)											3.2 (4/126)	3.2 (4/126)			10.3 (13/126)				1.7 (2/119)
Birds infested with positive species	27.9 (17/61)	3/1	1/1	1/1	0/1	1/3	5/6	2/5	3/1	0/1	0/1	7.1 (4/56)	3.6 (2/56)	0/2	0/1	0/1	30.4 (17/56)	4/4	8/8	0/1	3.9 (2/51)

[a] g3, g5, g6, and g8, *Bo. garinii* OspA types 3, 5, 6, and 8, respectively; vI and vII, *Bo. valaisiana* subtypes I and II, respectively.
[b] The numbers of infected larvae and nymphs are described in the text.
[c] Including coinfections (Fig. 1).
[d] NI, species not identified.

cies of the order Passeriformes. Among the 61 tick-infested birds, 12 species could be identified: blackbird (*Turdus merula*), blackcap (*Sylvia atricapilla*), blue tit (*Cyanistes caeruleus*), chiffchaff (*Phylloscopus collybita*), dunnock (*Prunella modularis*), Eurasian bullfinch (*Pyrrhula pyrrhula*), European robin (*Erithacus rubecula*), great tit (*Parus major*), marsh warbler (*Acrocephalus palustris*), reed warbler (*Acrocephalus scirpaceus*), song thrush (*Turdus philomelos*), and winter wren (*Troglodytes troglodytes*). The majority of bird species examined, with the exception of the great tit, are migrants or at least partial migrants. Most species were captured only in small numbers, so it was not possible to calculate reliable infestation rates for each species.

Among the small mammals, two species were identified: the yellow-necked mouse (*Apodemus flavicollis*) and the bank vole (*Myodes glareolus*), constituting the main part of the rodent fauna in the woodlands of Central Europe. The mean rate of infestation (MI) of voles with engorged ticks (107/73; MI = 1.5) was only marginally lower than that of mice (166/93; MI = 1.8) ($P = 0.07$). In total, rodents were significantly less infested with engorged stages of *I. ricinus* (273/166; MI = 1.5) than birds (211/61; MI = 3.5) ($P < 0.001$). Furthermore, birds were more frequently infested with nymphs (67.8%) than with larvae (32.2%), whereas the distribution for small mammals was the converse (larvae, 94.5%; nymphs, 4.8%; adults, 0.7%).

***Borrelia* infections.** Bird-feeding ticks (15.2%) were significantly more frequently infected with Lyme disease spirochetes than ticks collected off small mammals (2.6%; $P < 0.001$) and vegetation (5.1%; $P < 0.001$). Stages removed from birds showed the broadest range of *Borrelia* spp., including nine different OspA types (Table 1). The majority of *Borrelia* genospecies in bird-feeding *I. ricinus* ticks were *Borrelia garinii* OspA types (71.9%). Interestingly, in ticks from two blackbirds, one song thrush, and one robin, we identified *Borrelia afzelii* or *Borrelia bavariensis*, commonly considered to be rodent associated. Those species were even present in bird-feeding larvae (Table 1). The highest prevalence of *Borrelia* spp. was found in ticks removed from thrushes (*Turdus* spp.). Nearly half of the blackbirds (45.8%; 11/24) were infested with *Borrelia*-positive ticks, showing a prevalence of 22.8% (23/101). Ticks collected off song thrushes were also frequently infected (10.2%; 5/49). Due to limited sample sizes, *Borrelia* prevalence rates could not be calculated for ticks removed from other bird species.

Only two *Borrelia* spp., *Borrelia burgdorferi* and *Borrelia afzelii*, were detected in rodent-feeding ticks. The prevalence of Lyme disease spirochetes in larvae removed from small mammals was 0.8%, whereas bird-feeding larvae were considerably more frequently infected (20.6%) ($P < 0.001$). Multiple infections with different *Borrelia* spp. occurred only in bird-feeding ticks. Even two larvae, collected off a blackbird, were found to be triple infected (*Bo. garinii* OspA types 6 and 8 and *Bo. valaisiana* subtype I) and double infected (*Bo. garinii* OspA types 6 and 8) (Fig. 1).

Genospecies identification of *Borrelia*-positive amplicons from questing ticks resulted in *Bo. burgdorferi*, *Bo. bavariensis*, *Bo. garinii* OspA type 6, and *Bo. valaisiana* subtype I (Table 3). Subadult *I. ricinus* ticks (2.5%) were infected with borreliae considerably less frequently than adult stages (17.6%) ($P = 0.002$).

TABLE 2. Infection rates and species classification of pathogens in ticks collected off small mammals in Reifenstein (central Germany) from May to October 2007

Source	*Borrelia* spp.					*Rickettsia* spp.			*Babesia* spp.				Prevalence of *F. tularensis* (% [no. positive/no. tested])[a]
	Prevalence (% [no. positive/no. tested])	No. of infected larvae[b]/nymphs[b]/adults			Prevalence of *A. phagocytophilum* (% [no. positive/no. tested])[a]	Prevalence (% [no. positive/no. tested])	No. of infected larvae[b]/nymphs[b]/adults		Prevalence (% [no. positive/no. tested])	No. of infected larvae[b]/nymphs[b]/adults			
		Bo. burgdorferi	*Bo. afzelii*	NI[c]			*R. helvetica*	NI		*Ba. microti*	*Ba. divergens*	NI	
Feeding ticks	2.6 (7/273)				1.1 (3/273)	1.8 (5/273)			6.6 (18/273)				1.5 (4/264)
Larvae	0.8 (2/258)				1.2 (3/258)	1.9 (5/258)			5.4 (14/258)				1.6 (4/249)
Nymphs[d]	4/13				0/13	0/13			4/13				0/13
Adults[d]	1/2				0/2	0/2			0/2				0/2
Rodents infested	3.6 (6/166)	0/2/0	1/2/0	1/0/1	1.8 (3/166)	3.0 (5/166)	4/0/0	1/0/0	10.2 (17/166)	7/3/0	7/0/0	0/1/0	1.3 (2/158)
Rodents with positives													

[a] The numbers of infected larvae, nymphs, and adults are described in the text.
[b] Including coinfections (Fig. 1).
[c] NI, species not identified.
[d] Sample sizes were not sufficient for calculation of infection prevalence rates.

TABLE 3. Infection rates and species classification of pathogens in questing ticks collected in Reifenstein (central Germany) from May to October 2007

Source	*Borrelia* spp.						*Rickettsia* spp.			*Babesia* spp.			Prevalence of *F. tularensis* (% [no. positive/ no. tested])[a]
	Prevalence (% [no. positive/ no. tested])	No. of infected nymphs[b]/adults					Prevalence (% [no. positive/ no. tested])	No. of infected nymphs[b]/ adults		Prevalence (% [no. positive/ no. tested])	No. of infected nymphs[b]/adults		
		Bo. burgdorferi	*Bo. afzelii*	g6[c]	vI[d]	NI[e]		*R. helvetica*	NI		*Ba. microti*	*Ba. divergens*	
Ticks (total)	5.1 (10/196)						4.1 (8/196)			10.7 (21/196)			1.6 (3/192)
Nymphs	2.5 (4/162)						3.7 (6/162)			12.3 (20/162)			1.9 (3/159)
Adults	17.6 (6/34)	1/3	1/1	0/1	1/1	1/0	5.9 (2/34)	1/1	5/1	2.9 (1/34)	9/1	11/0	(0/33)

[a] The numbers of infected nymphs and adults are described in the text.
[b] Including coinfections (Fig. 1).
[c] g6, *Bo. garinii* OspA type 6.
[d] vI, *Bo. valaisiana* subtype I.
[e] NI, species not identified.

***Anaplasma phagocytophilum* infections.** Agents of human granulocytic anaplasmosis were rare in ticks collected off birds (3.2%) and rodents (1.1%). All positive amplicons were identified as *A. phagocytophilum*. Although 7.1% of the birds were carrying *Anaplasma*-infected ticks, only two out of six infections were found in bird-feeding larvae (Table 1). One of them was feeding on a robin, and the other one was feeding on a blackbird, together with a nymph infected with the same pathogen. In contrast to this, all *Anaplasma* infections in ticks removed from small mammals were detected in larvae feeding on different yellow-necked mice (Table 2). None of the host-seeking ticks was found to be infected with *A. phagocytophilum*.

***Rickettsia* infections.** Spotted fever group rickettsiae occurred in bird- and rodent-feeding ticks (2.1% and 1.8%, respectively) as well as in questing stages (4.1%). No significant differences in the prevalence of these agents could be observed between ticks from different sources ($P > 0.05$). Species identification of positive amplicons derived from bird-feeding ticks resulted in *R. monacensis* and *R. helvetica* (Table 1). Only blackbirds were parasitized by *Rickettsia*-positive ticks (8.3%). Small mammals were almost exclusively infested with *R. helvetica*-infected *I. ricinus* ticks.

***Francisella tularensis* infections.** *Francisella tularensis* was detected in *I. ricinus* ticks in Germany for the first time, showing prevalence rates of 1.2% for bird-feeding ticks, 1.5% for rodent-feeding ticks, and 1.6% for host-seeking ticks (Tables 1 to 3). One yellow-necked mouse carried three *F. tularensis*-infected larvae.

***Babesia* infections.** Parasites of the genus *Babesia* were frequently found in samples from all sources. The majority of *Babesia* infections were detected in bird-feeding ticks (13.2%) and questing ticks (10.7%). Ticks collected off rodents showed a *Babesia* prevalence rate of 6.6%. In all three groups, further classification revealed the presence of large and small *Babesia* spp. (*Ba. divergens* and *Ba. microti*). Feeding larvae removed from birds and small mammals were also infected with pathogenic babesiae and even with the species *Ba. microti*, which is not transovarially transmitted (Tables 1 and 2). Voles and mice were almost equally parasitized by *Babesia*-infected ticks (12.3% and 8.6%, respectively). Blackbirds were most frequently infested with *Babesia*-positive ticks (45.8%), showing a prevalence of 17.8%. One blackbird was parasitized by three *Babesia*-infected larvae (two *Ba. divergens*, one *Ba. microti*) and one positive nymph (*Ba. divergens*). On another blackbird,

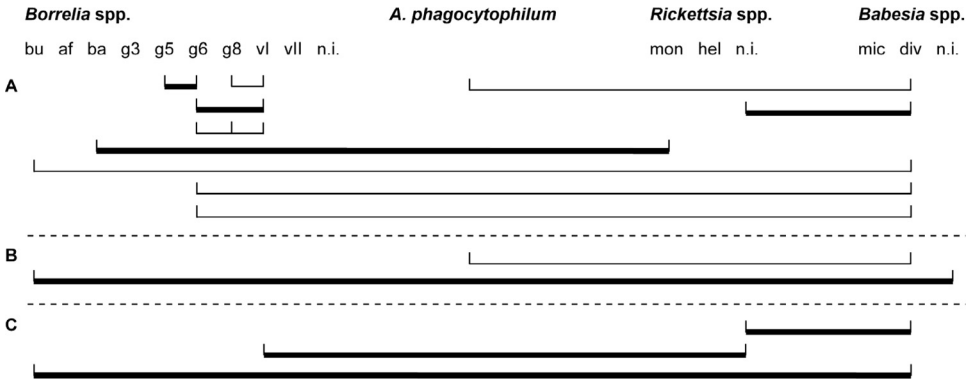

FIG. 1. Coinfections detected in bird-feeding (A), rodent-feeding (B), and host-seeking (C) *I. ricinus* ticks collected in Reifenstein (central Germany) from May to October 2007. Every horizontal line stands for one coinfected tick (thin lines, larvae; bold lines, nymphs). bu, *Bo. burgdorferi*; af, *Bo. afzelii*; ba, *Bo. bavariensis*; g3, g5, g6, and g8, *Bo. garinii* OspA types 3, 5, 6, and 8, respectively; vI and vII, *Bo. valaisiana* subtypes I and II, respectively; mon, *R. monacensis*; hel, *R. helvetica*; mic, *Ba. microti*; div, *Ba. divergens*.

three *Ba. divergens*-infected larvae were found. Positive 18S rRNA amplicons were also detected in ticks from bullfinch, dunnock, and marsh warbler, but due to the small sample size, prevalence rates were not calculated for each bird species.

Coinfections. A total of 10.9% (15/137) of all positive ticks were infected with at least two pathogens, and one-third of those (5/15) were coinfected with *Borrelia* and *Babesia* species. Other combinations were *Borrelia* spp. and *Rickettsia* spp. (2/15), *Babesia* spp. and *Rickettsia* spp. (2/15), *Babesia* spp. and *A. phagocytophilum* (2/15), and mixed infections with different *Borrelia* genospecies (4/15) (Fig. 1). The highest number of coinfections was found in bird-feeding pathogen-positive ticks (16.1%; 10/62; Fig. 1), all of which were collected off blackbirds. One blackbird carried subadult ticks that were altogether infected with six pathogens (*Bo. bavariensis*, *Bo. valaisiana* subtype II, *A. phagocytophilum*, *R. monacensis*, *R. helvetica*, *Ba. divergens*). Another three blackbirds were infested with pools of larvae infected with altogether three pathogens: *Bo. garinii* OspA type 6, *Bo. valaisiana* subtype I, and *Ba. microti*; *Bo. bavariensis*, *Ba. microti*, and *Ba. divergens*; and *Bo. burgdorferi*, *Bo. garinii* OspA type 6, and *Ba. divergens*).

The rate of coinfections in positive ticks collected off rodents was 6.6% (2/32). One yellow-necked mouse carried a larva infected with a combination of *A. phagocytophilum* and *Ba. divergens*. One nymph from a vole was found to be infected with *Bo. burgdorferi* and an unidentified *Babesia* species. Coinfections in host-seeking ticks occurred in 10.7% of the positive ticks (3/37), all of which were nymphs (Fig. 1).

DISCUSSION

Our data revealed that birds, in contrast to small mammals, constitute a major role as tick hosts in the investigation area. Other investigations have shown mean numbers of ticks per infested bird lower than the mean numbers found in the present study (3.5), reaching from 0.9 in Slovkia (50) to 2.6 in Sweden (5). A previous investigation in the area of Reifenstein resulted in a value of 2.5 ticks per infested bird (26). However, the mean rate of infestation strongly depends on the bird species included in the particular study. It is not surprising that ground-living birds show higher rates of infestation than other species. Even though they bear a high level of exposure to ticks, small mammals were less frequently infested with *I. ricinus* than birds. It has been shown that some rodent species are able to develop immunological resistance against tick antigens, leading to inefficient feeding and preterm detachment of ticks (7). In contrast, in the present study we could not observe significant differences in the mean rates of infestation with engorged ticks between mice and voles.

Because of the high prevalence of *Borrelia* spp. in feeding stages of *I. ricinus* (15.2%), ground-foraging passerines seem to be of major importance in the infectious cycle of Lyme disease spirochetes and constitute a key role in the maintenance of a multitude of *Borrelia* species. Other European studies reported *Borrelia* sp. infection rates of bird-feeding ticks ranging from 1.4% in Sweden (5) to 27.4% in Poland (9) and from 14.1% to 27.6% in Germany (14, 26, 54).

Birds are known to act as reservoir hosts for *Bo. burgdorferi*, *Bo. garinii*, and *Bo. valaisiana*, whereas other species, such as *Bo. afzelii* and *Bo. bavariensis*, were regarded as rodent associated (32). The fact that we found rodent-associated *Borrelia* spp. in bird-feeding ticks and even in larvae suggests that these associations might be not as strict as were previously thought. This is not the first time that *Bo. afzelii* has been detected in *I. ricinus* ticks feeding on birds (5, 26, 56) and even in larvae (9, 37, 42). In a study on a bird conservation island in the Baltic Sea, *Bo. afzelii* infections were evident in 11 out of 27 *Borrelia*-infected bird-feeding ticks, and 6 of them were larvae (14). Transovarial transmission of borreliae in *I. ricinus* is very rare (63), and due to the absence of other infected ticks, cofeeding transmission seems very unlikely in the above-mentioned samples. Thus, our data suggest that birds may also act as reservoirs for rodent-associated *Borrelia* species. The fact that bird DNA has previously been detected in *Bo. afzelii*-infected questing ticks supports this hypothesis (12, 41). Thrushes (*Turdus* spp.) in particular seem to be important reservoirs of various *Borrelia* spp., which has also been suggested in previous studies (5, 9, 14, 26).

The low prevalence of borreliae in rodent-feeding ticks (2.6%), particularly in larvae (0.8%), indicates that small mammals are not the main reservoirs for these pathogens, at least in the investigation area. Xenodiagnostic tests definitely proved the reservoir competence of rodents, but in most of the studies where high infection rates have been observed in rodent-feeding larvae, the samples were tested via immunofluorescence assay, involving a risk of cross-reactions (23, 33). Much lower infection rates in feeding larvae have been revealed in other studies, using the more specific PCR as the method of choice (16, 27, 44, 47). Considering our results and data from the literature, it can be assumed that in Europe, small mammals might be of less importance in the transmission cycle of Lyme disease spirochetes than was previously thought.

In contrast to other areas of central Germany, where incidences of up to 150 cases per 100,000 inhabitants have been observed, the incidence of human Lyme borreliosis in the investigation area was less than 40, which is in accordance with the relatively low prevalence of spirochetes in host-seeking ticks (5.1%). In comparison to the high prevalence of *Borrelia* spp. in bird-feeding ticks (15.2%), this suggests that some genospecies (e.g., *Bo. afzelii* and *Bo. garinii* OspA types 5 and 8; Tables 1 and 3) might fail in efficient transstadial transmission, because their expected prevalence in questing ticks should be at least as high as it is in subadult host-feeding stages. A more plausible explanation is that ticks parasitizing birds have a much higher rate of dispersal than rodent-feeding stages, which are less frequently infected. All examinations of bird-feeding ticks in a particular area represent only a snapshot of a current situation, and the majority of infected feeding ticks might drop off in a different habitat.

The rare occurrence of HGA agents in bird-feeding ticks (3.2%) might be the result of a reservoir incompetence of birds for this pathogen, as was supposed in other studies (1, 49). In contrast to this, a recent investigation revealed prevalence rates of up to 14.3% in bird-feeding larvae in Norway via quantitative PCR, whereas in Lithuania, none of the bird-feeding ticks tested positive for *A. phagocytophilum* (39). This suggests that birds act as reservoirs, and differences in the prevalence rates are probably caused by endemic occurrences of this agent. Rates of infection of questing ticks with *A. phagocytophilum* are also highly variable in different regions of Eu-

rope, reaching from 0% to 5.4% in central Germany (19) to 14.9% in Norway (45) and 23.6% in Denmark (48), supporting this hypothesis.

All *Anaplasma*-infected ticks from small mammals were larvae. Although the total prevalence was low (1.1%), rodents might be able to transmit these agents to ticks. This pathogen has already been detected in rodent tissue and feeding ticks (31, 53), but the duration of infectivity to ticks is low due to the acute course of anaplasmosis in these hosts (2). Furthermore, there is evidence that rodents develop immunity after infection with *A. phagocytophilum*, leading to a decrease of infectivity and the efficiency of cofeeding transmission (30). This explains their limited reservoir competence for HGA agents. In contrast to our results, investigations from the United Kingdom and Switzerland showed that voles are more frequently infested with *Anaplasma*-positive ticks than mice (4, 31). In the present study, the differences between the two species were not significant ($P > 0.05$), possibly because the prevalence of this agent in rodent-feeding ticks was very low.

Here, we report for the first time the occurrence of *R. monacensis* and *R. helvetica* in bird-feeding ticks in central Germany. The knowledge about the role of birds in the transmission cycle of SFG rickettsiae is still poor. In the Carpathians, only one bird-feeding nymph was found to be infected (50), whereas in Sweden a prevalence of 11.3% has recently been detected (11). We also found both species in 7.3% of ticks feeding on birds on an island in the Baltic Sea (18). Because the prevalence rate in the present study was low (2.1%) and due to the possibility of transovarial transmission, we cannot give solid evidence for a reservoir role for birds. Further investigations, including xenodiagnostic tests, are necessary to assess the role of birds as possible reservoirs for pathogenic rickettsiae. Nevertheless, we can at least assume that passerines act as vehicles and are therefore of importance in the dispersal of these bacteria. We also found *R. helvetica* in a small number of rodent-feeding ticks (1.8%). Data available so far on the role of rodents in the transmission cycle of rickettsiae are mainly based on seroprevalence studies, with seroprevalence rates of rodents ranging up to 9.1% (62). *R. helvetica* was recently reported in rodent-feeding ticks (larvae, 2.1%; nymphs, 8%) from Poland (51). Thus, our results represent the first detection of these pathogens in ticks feeding on rodents in Germany. However, no conclusions on the reservoir competence of small mammals can be drawn from our data, which indicates the need for further investigations. Only xenodiagnostic tests with sterile larvae and rickettsiemic rodents can fill this gap in knowledge.

The observed prevalence of SFG rickettsiae in host-seeking ticks (4.1%) is within the range of data from Germany, where infection rates of 0.4% up to 14.2% have been detected (8, 17, 41). A recent study in Thuringia reported a prevalence of 14.7% in questing ticks (19), suggesting that these agents are also heterogeneously spread in Germany.

Francisella tularensis was found in host-feeding and questing *I. ricinus* stages for the first time in Germany. Occasional records of *F. tularensis* in questing ticks have been reported from Austria (52), Switzerland (61), Serbia (34), and Czechoslovakia (22). These agents can cause natural infections in a wide range of animal species and can be transmitted by a large number of arthropods (40). Infections in humans are usually caused by contact with infected lagomorphs (hares and wild rabbits), but rodents are also an important reservoir. In a recent study in Germany, 386 small mammals and 432 ectoparasites were tested with PCR for the presence of *F. tularensis* DNA. The infected species were bank voles (*Myodes glareolus*), water voles (*Arvicola terrestris*), field voles (*Microtus agrestis*), common voles (*Microtus arvalis*), and yellow-necked mice (*Apodemus flavicollis*). The overall carrier rate was 4.9%, but none of the ectoparasites was positive (25). Birds may play a role as disseminators of the bacteria over large distances. Raptors, corvids, and gulls are usually resistant to infection with *F. tularensis* (unless they are immunodeficient due to starvation), but they can excrete the bacteria with their feces (35). Infected animals and carcasses may be a source of infection for these birds, but *Haemaphysalis* ticks, which can be found on rodents and lagomorphs as well as on birds, might also play a role in the transmission between these animals. *Ixodes ricinus* can harbor up to 10^7 cells of *F. tularensis* after feeding on terminally ill experimentally infected mice. A high degree of positivity was maintained for 1 month, and transstadial transmission was confirmed more than 1 year after infection (60). This indicates that ticks can maintain a natural reservoir over long periods of time.

The results of the present study (Table 1) support the assumption that birds also act as reservoir hosts for pathogenic babesiae (18). The life cycle of *Babesia* spp. is very complex, and so far there is no evidence that cofeeding is a possible way of transmission for this organism. At least for small *Babesia* spp., which are not transmitted transovarially by *I. ricinus* (15), a reservoir function of birds can be assumed, because one-half of the *Ba. microti*-specific amplicons were detected in feeding larvae. For large babesiae (e.g., *Ba. divergens*), where this form of transmission is present, we can at least assign birds an important role in the dispersal of *Babesia*-infected ticks.

Although *Babesia* infections have already been detected in tissues of several rodent species (24, 46), the question of whether these hosts are infective for parasitizing ticks could not be answered due to a lack of xenodiagnostic investigations. Here, we report for the first time the occurrence of *Ba. microti* and *Ba. divergens* in rodent-feeding ticks (6.6%), particularly in larvae (5.4%). By providing this missing link in the transmission cycle of pathogenic babesiae, we give solid evidence for a reservoir role of small mammals, at least for *Ba. microti*-like species. The fact that these agents were also present in a high number of questing ticks (10.7%) indicates that the risk of infection with pathogenic *Babesia* spp. in the investigation area is relatively high. A slightly lower prevalence of 5.0% has been detected in host-seeking ticks from another area in central Germany (20), but the spread of babesiae in Europe shows a high degree of variability. In Norway, only 0.9% of questing ticks were infected with these agents (43), whereas in Austria, a prevalence of 51.7% has been detected (3).

In view of the frequency (10.9%) and heterogeneity of coinfections detected in the present study, it can be assumed that the risk of multiple infections for humans and animals is relatively high, at least in the investigation area. Coinfections are common among Lyme disease patients, often leading to severe clinical manifestations and problems in diagnosis and treatment (55). Multiple infections of feeding larvae, e.g., with different *Borrelia* spp., indicate that the hosts were coinfected

too. The fact that some blackbirds carried pools of larvae that were altogether infected with multiple pathogens not transmitted transovarially supports this assumption. Birds, particularly blackbirds, seem to be a pivotal element for the conjunction of the transmission cycles of different tick-borne pathogens. In a recent study, we also found coinfected ticks on other bird species, e.g., European robins (13).

In the present study we provide interesting insights into the occurrence and cocirculation of established and emerging tick-borne pathogens in a single natural habitat of central Germany. Furthermore, we extend the state of knowledge on the reservoir competence of several bird and rodent species and their role in the dispersal of these agents. Nevertheless, many ecological issues, especially regarding the biological fundamentals for host-pathogen relationships, still remain unsolved. The temporal and regional heterogeneities in the occurrence of pathogens in host-feeding and questing ticks are a result of interactions between ticks, reservoir hosts, and nonreservoir hosts, each of which is affected by a multitude of biotic and abiotic factors. These interactions are very complex and difficult to explore. Although our investigations took place in a relatively small area in central Germany, our findings will most probably also be observed in other areas of endemicity because the host species examined are widely distributed all over Europe. Therefore, wide-ranging investigations on possible reservoir hosts, including the measurement of climate parameters, are indispensable for the reliable assessment of the risk of tick-borne diseases.

ACKNOWLEDGMENT

We thank Andreas Goedecke of the ProRing Association for collecting the bird-feeding ticks.

REFERENCES

1. **Alekseev, A. N., H. V. Dubinina, A. V. Semenov, and C. V. Bolshakov.** 2001. Evidence of ehrlichiosis agents found in ticks (Acari: Ixodidae) collected from migratory birds. J. Med. Entomol. **38:**471–474.
2. **Beugnet, F., and J. L. Marie.** 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. **163:**298–305.
3. **Blaschitz, M., M. Narodoslavsky-Gfoller, M. Kanzler, G. Stanek, and J. Walochnik.** 2008. *Babesia* species occurring in Austrian *Ixodes ricinus* ticks. Appl. Environ. Microbiol. **74:**4841–4846.
4. **Bown, K. J., M. Begon, M. Bennett, Z. Woldehiwet, and N. H. Ogden.** 2003. Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. Emerg. Infect. Dis. **9:**63–70.
5. **Comstedt, P., S. Bergstrom, B. Olsen, U. Garpmo, L. Marjavaara, H. Mejlon, A. G. Barbour, and J. Bunikis.** 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerg. Infect. Dis. **12:**1087–1095.
6. **Derdáková, M., and D. Lenčáková.** 2005. Association of genetic variability within the *Borrelia burgdorferi* sensu lato with the ecology, epidemiology of Lyme borreliosis in Europe. Ann. Agric. Environ. Med. **12:**165–172.
7. **Dizij, A., and K. Kurtenbach.** 1995. *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. Parasite Immunol. **17:**177–183.
8. **Dobler, G., and R. Wölfel.** 2009. Typhus and other rickettsioses: emerging infections in Germany. Dtsch. Ärztebl. Int. **106:**348–354. (In German.)
9. **Dubska, L., I. Literak, E. Kocianova, V. Taragelová, and O. Sychra.** 2009. Differential role of passerine birds in distribution of *Borrelia* spirochetes, based on data from ticks collected from birds during the postbreeding migration period in Central Europe. Appl. Environ. Microbiol. **75:**596–602.
10. **Dumler, J. S., A. F. Barbet, C. P. Bekker, G. A. Dasch, G. H. Palmer, S. C. Ray, Y. Rikihisa, and F. R. Rurangirwa.** 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. **51:**2145–2165.
11. **Elfving, K., B. Olsen, S. Bergstrom, J. Waldenstrom, A. Lundkvist, A. Sjostedt, H. Mejlon, and K. Nilsson.** 2010. Dissemination of spotted fever rickettsia agents in Europe by migrating birds. PLoS One **5:**e8572.
12. **Estrada-Peña, A., J. J. Osacar, B. Pichon, and J. S. Gray.** 2005. Hosts and pathogen detection for immature stages of *Ixodes ricinus* (Acari: Ixodidae) in north-central Spain. Exp. Appl. Acarol. **37:**257–268.
13. **Franke, J., F. Meier, A. Moldenhauer, E. Straube, W. Dorn, and A. Hildebrandt.** Established and emerging pathogens in *Ixodes ricinus* ticks collected from birds on a conservation island in the Baltic Sea. Med. Vet. Entomol., in press.
14. **Franke, J., A. Moldenhauer, A. Hildebrandt, and W. Dorn.** 2010. Are birds reservoir hosts for *Borrelia afzelii*? Ticks Tick-Borne Dis. **1:**109–112.
15. **Gray, J., A. Zintl, A. Hildebrandt, K. P. Hunfeld, and L. Weiss.** 2010. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. Ticks Tick-Borne Dis. **1:**3–10.
16. **Gray, J. S., F. Kirstein, J. N. Robertson, J. Stein, and O. Kahl.** 1999. *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks and rodents in a recreational park in south-western Ireland. Exp. Appl. Acarol. **23:**717–729.
17. **Hartelt, K., R. Oehme, H. Frank, S. O. Brockmann, D. Hassler, and P. Kimmig.** 2004. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in southern Germany. Int. J. Med. Microbiol. **293**(Suppl. 37)**:**86–92.
18. **Hildebrandt, A., J. Franke, F. Meier, S. Sachse, W. Dorn, and E. Straube.** 2010. The potential role of migratory birds in transmission cycles of *Babesia* spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp. Ticks Tick-Borne Dis. **1:**105–107.
19. **Hildebrandt, A., A. Krämer, S. Sachse, and E. Straube.** 2010. Detection of *Rickettsia* spp. and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in a region of Middle Germany (Thuringia). Ticks Tick-Borne Dis. **1:**52–56.
20. **Hildebrandt, A., K. Pauliks, S. Sachse, and E. Straube.** 26 April 2010, posting date. Coexistence of *Borrelia* spp. and *Babesia* spp. in *Ixodes ricinus* ticks in Middle Germany. Vector Borne Zoonotic Dis. doi:10.1089/vbz.2009.0202.
21. **Hildebrandt, A., K. H. Schmidt, V. Fingerle, B. Wilske, and E. Straube.** 2002. Prevalence of granulocytic ehrlichiae in *Ixodes ricinus* ticks in Middle Germany (Thuringia) detected by PCR and sequencing of a 16S ribosomal DNA fragment. FEMS Microbiol. Lett. **211:**225–230.
22. **Hubalek, Z., and J. Halouzka.** 1997. Mosquitoes (Diptera: Culicidae), in contrast to ticks (Acari: Ixodidae), do not carry *Francisella tularensis* in a natural focus of tularemia in the Czech Republic. J. Med. Entomol. **34:**660–663.
23. **Humair, P. F., N. Turrian, A. Aeschliemann, and L. Gern.** 1993. *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. Folia Parasitol. **40:**65–70.
24. **Hunfeld, K.-P., A. Hildebrandt, and J. S. Gray.** 2008. Babesiosis: recent insights into an ancient disease. Int. J. Parasitol. **38:**1219–1237.
25. **Kaysser, P., E. Seibold, K. Matz-Rensing, M. Pfeffer, S. Essbauer, and W. D. Splettstoesser.** 2008. Re-emergence of tularemia in Germany: presence of *Francisella tularensis* in different rodent species in endemic areas. BMC Infect. Dis. **8:**157.
26. **Kipp, S., A. Goedecke, W. Dorn, B. Wilske, and V. Fingerle.** 2006. Role of birds in Thuringia, Germany, in the natural cycle of *Borrelia burgdorferi* sensu lato, the Lyme disease spirochaete. Int. J. Med. Microbiol. **296**(Suppl. 1)**:** 125–128.
27. **Kurtenbach, K., M. Peacey, S. G. T. Rijpkema, N. Hoodless, P. A. Nuttall, and S. E. Randolph.** 1998. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. Appl. Environ. Microbiol. **64:**1169–1174.
28. **Ladbury, G. A., S. Stuen, R. Thomas, K. J. Bown, Z. Woldehiwet, E. G. Granquist, K. Bergstrom, and R. J. Birtles.** 2008. Dynamic transmission of numerous *Anaplasma phagocytophilum* genotypes among lambs in an infected sheep flock in an area of anaplasmosis endemicity. J. Clin. Microbiol. **46:**1686–1691.
29. **Lenčáková, D., C. Hizo-Teufel, B. Petko, U. Schulte-Spechtel, M. Stanko, B. Wilske, and V. Fingerle.** 2006. Prevalence of *Borrelia burgdorferi* s.l. OspA types in *Ixodes ricinus* ticks from selected localities in Slovakia and Poland. Int. J. Med. Microbiol. **296**(Suppl. 40)**:**108–118.
30. **Levin, M. L., and D. Fish.** 2000. Immunity reduces reservoir host competence of *Peromyscus leucopus* for *Ehrlichia phagocytophila*. Infect. Immun. **68:**1514–1518.
31. **Liz, J. S., L. Anderes, J. W. Sumner, R. F. Massung, L. Gern, B. Rutti, and M. Brossard.** 2000. PCR detection of granulocytic ehrlichiae in *Ixodes ricinus* ticks and wild small mammals in western Switzerland. J. Clin. Microbiol. **38:**1002–1007.
32. **Margos, G., S. A. Vollmer, M. Cornet, M. Garnier, V. Fingerle, B. Wilske, A. Bormane, L. Vitorino, M. Collares-Pereira, M. Drancourt, and K. Kurtenbach.** 2009. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl. Environ. Microbiol. **75:**5410–5416.
33. **Michalik, J., T. Hofman, A. Buczek, M. Skoracki, and B. Sikora.** 2003. *Borrelia burgdorferi* s.l. in *Ixodes ricinus* (Acari: Ixodidae) ticks collected from vegetation and small rodents in recreational areas of the city of Poznań. J. Med. Entomol. **40:**690–697.
34. **Milutinović, M., T. Masuzawa, S. Tomanović, Z. Radulović, T. Fukui, and Y.**

Okamoto. 2008. *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Francisella tularensis* and their infections in host-seeking *Ixodes ricinus* ticks collected in Serbia. Exp. Appl. Acarol. **45:**171–183.
35. **Mörner, T., and R. Mattsson.** 1988. Experimental infection of five species of raptors and of hooded crows with *Francisella tularensis* biovar palaearctica. J. Wildl. Dis. **24:**15–21.
36. **Nilsson, K., O. Lindquist, A. J. Liu, T. G. Jaenson, G. Friman, and C. Pahlson.** 1999. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. J. Clin. Microbiol. **37:**400–403.
37. **Olsen, B., T. Jaenson, and S. Bergstrom.** 1995. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. Appl. Environ. Microbiol. **61:**3082–3087.
38. **Parola, P., C. D. Paddock, and D. Raoult.** 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev. **18:**719–756.
39. **Paulauskas, A., J. Radzijevskaja, and O. Rosef.** 2009. *Anaplasma* in ticks feeding on migrating birds and questing ticks in Lithuania and Norway. Clin. Microbiol. Infect. **15:**34–39.
40. **Petersen, J. M., P. S. Mead, and M. E. Schriefer.** 2009. *Francisella tularensis*: an arthropod-borne pathogen. Vet. Res. **40:**7.
41. **Pichon, B., O. Kahl, B. Hammer, and J. S. Gray.** 2006. Pathogens and host DNA in *Ixodes ricinus* nymphal ticks from a German forest. Vector Borne Zoonotic Dis. **6:**382–387.
42. **Poupon, M.-A., E. Lommano, P. F. Humair, V. Douet, O. Rais, M. Schaad, L. Jenni, and L. Gern.** 2006. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. Appl. Environ. Microbiol. **72:**976–979.
43. **Radzijevskaja, J., A. Paulauskas, and O. Rosef.** 2008. Prevalence of *Anaplasma phagocytophilum* and *Babesia divergens* in *Ixodes ricinus* ticks from Lithuania and Norway. Int. J. Med. Microbiol. **298**(Suppl. 1)**:**218–221.
44. **Ragagli, C., L. Bertolotti, M. Giacobini, A. Mannelli, D. Bisanzio, G. Amore, and L. Tomassone.** 21 May 2010, posting date. Transmission dynamics of *Borrelia lusitaniae* and *Borrelia afzelii* among *Ixodes ricinus*, lizards, and mice in Tuscany, Central Italy. Vector Borne Zoonotic Dis. doi:10.1089/vbz.2008.0195.
45. **Rosef, O., A. Paulauskas, and J. Radzijevskaja.** 2009. Prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing Ixodes ricinus ticks in relation to the density of wild cervids. Acta Vet. Scand. **51:**47.
46. **Siński, E., A. Bajer, R. Welc, A. Pawełczyk, M. Ogrzewalska, and J. M. Behnke.** 2006. *Babesia microti*: prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes district of north-eastern Poland. Int. J. Med. Microbiol. **296**(Suppl. 1)**:**137–143.
47. **Siński, E., A. Pawełczyk, A. Bajer, and J. Behnke.** 2006. Abundance of wild rodents, ticks and environmental risk of Lyme borreliosis: a longitudinal study in an area of Mazury Lakes district of Poland. Ann. Agric. Environ. Med. **13:**295–300.
48. **Skarphedinsson, S., B. F. Lyholm, M. Ljungberg, P. Sogaard, H. J. Kolmos, and L. P. Nielsen.** 2007. Detection and identification of *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Rickettsia helvetica* in Danish *Ixodes ricinus* ticks. APMIS **115:**225–230.
49. **Skotarczak, B., A. Rymaszewska, B. Wodecka, M. Sawczuk, M. Adamska, and A. Maciejewska.** 2006. PCR detection of granulocytic *Anaplasma* and *Babesia* in *Ixodes ricinus* ticks and birds in West-Central Poland. Ann. Agric. Environ. Med. **13:**21–23.
50. **Spitalska, E., I. Literak, O. A. Sparagano, M. Golovchenko, and E. Kocianova.** 2006. Ticks (Ixodidae) from passerine birds in the Carpathian region. Wien Klin. Wochenschr. **118:**759–764.
51. **Stańczak, J., M. Racewicz, J. Michalik, S. Cieniuch, B. Sikora, and M. Skoracki.** 2010. Prevalence of infection with *Rickettsia helvetica* in feeding ticks and their hosts in western Poland. Clin. Microbiol. Infect. **15**(Suppl. 2)**:**328–329.
52. **Stanek, G.** 2009. Pandora's box: pathogens in *Ixodes ricinus* ticks in Central Europe. Wien Klin. Wochenschr. **121:**673–683. (In German.)
53. **Štefančíková, A., M. Derdáková, D. Lenčáková, R. Ivanová, M. Stanko, L. Čisláková, and B. Petko.** 2008. Serological and molecular detection of *Borrelia burgdorferi* sensu lato and Anaplasmataceae in rodents. Folia Microbiol. (Praha) **53:**493–499.
54. **Stern, C., A. Kaiser, W. A. Maier, and H. Kampen.** 2006. Die Rolle von Amsel (*Turdus merula*), Rotdrossel (*Turdus iliacus*) und Singdrossel (*Turdus philomelos*) als Blutwirte für Zecken (Acari: Ixodidae) und Reservoirwirte für vier Genospezies des *Borrelia burgdorferi*-Artenkomplexes. Mitt. Dtsch. Ges. Allg. Angew. Ent. **15:**349–356.
55. **Swanson, S. J., D. Neitzel, K. D. Reed, and E. A. Belongia.** 2006. Coinfections acquired from *Ixodes ticks*. Clin. Microbiol. Rev. **19:**708–727.
56. **Taragelová, V., J. Koči, K. Hanincová, K. Kurtenbach, M. Derdáková, N. H. Ogden, I. Literak, E. Kocianová, and M. Labuda.** 2008. Blackbirds and song thrushes constitute a key reservoir of *Borrelia garinii*, the causative agent of borreliosis in Central Europe. Appl. Environ. Microbiol. **74:**1289–1293.
57. **Tärnvik, A., H. S. Priebe, and R. Grunow.** 2004. Tularaemia in Europe: an epidemiological overview. Scand. J. Infect. Dis. **36:**350–355.
58. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876–4882.
59. **Versage, J. L., D. D. Severin, M. C. Chu, and J. M. Petersen.** 2003. Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. J. Clin. Microbiol. **41:**5492–5499.
60. **Výrosteková, V.** 1994. Transstadial transmission of *Francisella tularensis* by *Ixodes ricinus* ticks infected during the nymphal stage. Epidemiol. Mikrobiol. Imunol. **43:**166–170. (In Slovak.)
61. **Wicki, R., P. Sauter, C. Mettler, A. Natsch, T. Enzler, N. Pusterla, P. Kuhnert, G. Egli, M. Bernasconi, R. Lienhard, H. Lutz, and C. M. Leutenegger.** 2000. Swiss Army survey in Switzerland to determine the prevalence of *Francisella tularensis*, members of the *Ehrlichia phagocytophila genogroup*, *Borrelia burgdorferi* sensu lato, and tick-borne encephalitis virus in ticks. Eur. J. Clin. Microbiol. Infect. Dis. **19:**427–432.
62. **Zhan, L., W. C. Cao, C. Y. Chu, B. G. Jiang, F. Zhang, W. Liu, J. S. Dumler, X. M. Wu, S. Q. Zuo, P. H. Zhang, H. N. Huang, Q. M. Zhao, N. Jia, H. Yang, J. H. Richardus, and J. D. Habbema.** 2009. Tick-borne agents in rodents, China, 2004–2006. Emerg. Infect. Dis. **15:**1904–1908.
63. **Zhioua, E., A. Aeschlimann, and L. Gern.** 1994. Infection of field-collected *Ixodes ricinus* (Acari: Ixodidae) larvae with *Borrelia burgdorferi* in Switzerland. J. Med. Entomol. **31:**763–766.